



Histamine as biogenic amine residue in imported frozen fish

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ABSTRACT

A total of ninety random samples of imported frozen fish represented by Mackerel, Herring and Saurus (30 of each) from two different countries namely A and B (15 samples of each) were collected from different fish markets in Menoufiya governorate. Each sample was weighed about 150 g and stored at -18°C. The collected samples were packed separately in sterile polyethylene bag and transferred directly to the laboratory with minimum delay for determination of their toxic residues. The obtained results recorded that the occurrence of histamine in both country (A) and in country (B) were 100% in the Mackerel, Herring and Saurus samples. The mean value of histamine in the country (A) samples were 38.14 ± 2.93, 31.67 ± 2.15 and 19.05 ± 1.68 mg/kg in the Mackerel, Herring and Saurus, respectively. The average concentration of histamine in country (B) samples were 33.96 ± 2.45, 24.51 ± 1.72 and 12.83 ± 1.19 mg/kg in the Mackerel, Herring and Saurus, respectively. It could be inferred that regarding the products contamination, the highest histamine contamination was in mackerel followed by herring then saurus. Regarding the locality, country (A) represented the highest contamination of histamine followed by country (B).

Keywords: Mackerel, Herring, Saurus, histamine

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1. INTRODUCTION

Biogenic amines can be used as quality index, once they are formed by bacterial activity and are resistant to thermal treatment, thus reflecting the hygienic conditions and the of quality raw material of food processing (Sagrati et al., 2012). Toxicological levels of biogenic amines especially histamine is very complicated to establish because it bases on individual characteristics and the presence of other amine, however, a maximum total biogenic amine levels of 750-900 ppm has been proposed (Ladero et al., 2010). The content of histamine differs according to fish species. For example, Scombridae family, such as Tuna, sardine and Bonito are characterized by the presence of high levels of free histamine in their muscle, also according to the season of the year, environment, genetics, sex, food, storage period and sampled tissue. Poisoning with histamine can lead to toxicological effects to consumers such as pseudo-allergic reaction, diarrhea or spasm of bronchi, urticaria. The content of histamine is considered as a criterion of the fish quality (Fletcher et al., 1998). Also symptoms of histamine toxicity include vomiting, abdominal cramps, perspiration, flushing, headaches and burning sensations in the mouth (Taylor and Bush, 1988). Biogenic amines

may be considered as carcinogenic because of their ability to react with nitrites to form potentially carcinogenic nitrosamines (Shalaby, 1996). While histamine is linked with the deterioration of fish in general, it is not always found in spoiled fish, so cannot be considered as an indicator of the deterioration of stored fish (Heidi and Collin, 2005).

The aim of the present work was to determine histamine (biogenic amine) in imported frozen fish which could be indicative to its healthiness for human consumption.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 90 random samples of imported frozen fish represented by Mackerel, Herring and Saurus (30 of each) from two different countries namely A and B (15 samples of each fish species for each country) were collected from different fish markets in Menoufiya governorate.

2.2. Determination of biogenic amines:

Histamine as one of biogenic amines was determined in all examined samples according to

the protocol recommended by Krause (Krause et al., 1995) et al. (1995) and Pinho (Pinho et al., 2001) et al. (2001).

2.2.1. Reagents preparation:

- Dansyl chloride solution: 500mg of dansyl chloride were dissolved in 100 ml acetone.
- Standard solutions: Stock standard solutions of the tested amines were prepared as the following: add 25 mg of each standard pure amine (histamine-2HCl) was dissolved in 25 ml distilled water.

2.2.2. Extraction of samples and formation of dansylamines (Armağan, 2006):

One hundred µl stock standard solution (or sample extract) was transferred to 50ml vial and dried under vacuum. About 0.5 ml of saturated NaHCO₃ solution was added to the residue of the sample extract (or the standard). Vial was stoppered and carefully mixed to prevent loss due to spattering. Carefully, 1.0 ml dansyl chloride solution was added and mixed thoroughly using vortex mixer. The reaction mixture was incubated at 55°C for 45 min. About 10 ml of distilled water were added to the reaction mixture, then vial was stoppered and shaken vigorously using vortex mixer. The extraction of dansylated biogenic amine was carried out using 5ml of diethyl ether for 3 times. Again vial was stoppered, shaken for 11.0 min and the ether layers were collected in a culture tube using disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35°C in dry bath with aid of current air. The obtained dry material was dissolved in 1ml methanol and 10µl were injected in High performance liquid chromatography (HPLC). Data were integrated and recorded using Chemstation Software program.

3. RESULTS

The obtained results in table (1) revealed that the mean values of histamine in the country (A) samples were 38.14 ± 2.93 , 31.67 ± 2.15 and 19.05 ± 1.68 mg% in the Mackerel, Herring and Saurus, respectively. The average concentration of histamine in country (B) samples were 33.96 ± 2.45 , 24.51 ± 1.72 and 12.83 ± 1.19 mg% in the Mackerel, Herring and Saurus, respectively. Furthermore, in country (A), the percentages of unaccepted samples in table (2) were 66.67, 53.33 and 46.67 % in the Mackerel, Herring and Saurus samples, respectively. In country (B), the percentages of unaccepted samples were 53.33, 53.33 and 26.67% in the Mackerel, Herring and Saurus samples, respectively.

Table (1): Statistical analytical results of histamine levels (mg %) in the examined samples of imported frozen fish. (n=15)

| | | +ve samples | | | | | |
|---------------------|----|-------------|------|------|------------------|--|--|
| country / fish | | Min. | Max. | Mean | ± S.E* | | |
| | | No | % | | | | |
| <u>country (A):</u> | | | | | | | |
| Mackerel | 15 | 100 | 6.1 | 72.8 | 38.14 ± 2.93 | | |
| Herring | 15 | 100 | 4.9 | 60.3 | 31.67 ± 2.15 | | |
| saurus | 15 | 100 | 2.5 | 37.6 | 19.05 ± 1.68 | | |
| <u>country (B):</u> | | | | | | | |
| Mackerel | 15 | 100 | 5.4 | 63.2 | 33.96 ± 2.45 | | |
| Herring | 15 | 100 | 3.6 | 44.9 | 24.51 ± 1.72 | | |
| saurus | 15 | 100 | 1.9 | 25.7 | 12.83 ± 1.19 | | |

S.E*= Standard error of mean

Table (2): Acceptability of the examined samples of imported frozen fish based on their levels of histamine. (n=15)

| Country | Maximum Permissible Limit (mg/kg)* | Unaccepted No. | MacMackerel % | Unaccepted No. | Herring % | Unaccepted No. | saurus % |
|---------------------|------------------------------------|----------------|---------------|----------------|-----------|----------------|----------|
| <u>country (A):</u> | 20 | 10 | 66.67 | 8 | 53.33 | 7 | 46.67 |
| <u>country (B):</u> | 20 | 8 | 53.33 | 5 | 53.33 | 4 | 26.67 |
| Total (45) | 20 | 18 | 120.00 | 13 | 106.66 | 11 | 73.34 |

* Egyptian Organization for Standardization and Quality control "EOS" (2005)

4. DISCUSSION

Biogenic amines act as the main cause of some toxic symptoms, such as headache, migraine,

diarrhea, hypotension and unconsciousness and cardiac arrhythmia in severe cases. The most important one of them, is histamine which causes the most serious physical problems, for example, rashes, sweating and respiratory disorders (Wigand et al., 2012). Furthermore, it was found that the human tolerance limit for histamine is 10 mg per 100gm (Håstein et al., 2006). A high histamine content in fish flesh has long been considered the most probable result of this type of food poisoning (Scombroid food poisoning) (Anonymous, 1991).

The current results in histamine relatively agree with those recorded by El-Sayed (2010) who recorded that the mean value of histamine in fresh water fish, canned fish, pickled fish, smoked fish samples were 8.35 ± 0.5 , 7.24 ± 0.39 , 6.91 ± 0.46 and 10.62 ± 0.70 ppm, respectively also results agree with El-Shorbagy et al. (2000); Galarini et al. (1996). In contrast, our results disagree with those recorded by Walaa (2016) who found that the means of histamine in the imported frozen examined samples of Mackerel, Sardine, Saurus and Barboni fish were 53.28 ± 2.13 , 29.65 ± 1.41 , 22.81 ± 1.06 and 16.03 ± 0.94 ppm, respectively also results disagree with Samaha et al. (1997) and Azudine and Sarri (1988) According to Egyptian Organization of Standardization EOS (2005) which recommended that maximal permissible limits for histamine was 20 (mg/100g) in fish, the number of un-accepted samples in country(A) are 10, 8 and 7 represented as 66.67%, 53.33% and 46.67% While, in country(B) the number of un-accepted samples are 8, 5 and 4 represented as 53.33%, 33.33% and 26.67% in the examined mackerel fish, herring fish and saurus fish, respectively (Table, 2).

From the obtained results, the collected imported frozen fish samples (Mackerel, Herring and Saurus) were highly contaminated with toxic biogenic amine residue (histamine). Regarding the products contamination, the highest histamine contamination was in Mackerel followed by Herring then Saurus. It is recommended that Frozen Saurus is the lowest in fish samples histamine content. So, it is the best fish of choice. Periodical inspection and control of markets, fish should be practiced to confirm fish safety. Continuous screening of frozen fish for biogenic amines in local markets and restaurants should be occurred under veterinary supervision.

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